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EXPERIMENTAL STUDY

Effect of Yiqiyangyin recipe on heparanase and nephrin in rats with adriamycin-induced nephropathy

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Abstract

OBJECTIVE: To discuss the mechanism of Yiqiyangyin recipe in rats with adriamycin (ADR)-induced nephropathy.

METHODS: We randomly divided 30 Sprague-Dawley rats into 5 groups: control, model, glucocorticoid, Chinese herb, and Chinese herb plus glucocorticoid groups. The 24-h urine volume was collected on days 7, 14, 21, and 28 after ADR injection, and all rats were killed on day 28. We measured the 24-h levels of urinary protein, serum cholesterol, and serum triglycerides, and renal function of all rats by using routine biochemical methods. Pathological changes in the rat kidneys were observed under light and electron microscopes. Heparanase (HPA) mRNA expression levels were measured using real-time fluorescence-quantitative polymerase chain reaction. Urine levels of HPA in all groups were measured using enzyme-linked immunosorbent assay. The expression of nephrin was detected by immunohistochemical staining and quantitatively analyzed using Motic image analysis 3.2 software.

RESULTS: The levels of urinary protein and serum triglycerides and cholesterol in rats with ADR-induced nephropathy increased on day 14. The serum albumin levels simultaneously decreased. All the changes reached the peak on day 28. Examination under the light microscope showed inflammatory cells and slight fibroplasia in the renal interstitium in the model group, but fewer inflammatory cells were observed in the intervention groups than those in the model group. Examination under the electron microscope showed extensive fusion of foot processes in ADR rats. HPA mRNA expression was higher in the model group than that in the control group. The HPA mRNA levels in the intervention groups, especially in the Chinese herb group, and Chinese herb plus glucocorticoid group were significantly lower than the level in the model group. The HPA expression levels correlated significantly with the proteinuria level. No significant difference was found in the HPA level in urine between the intervention groups and the model group, whereas the model group had a higher urinary HPA level than the control group. Nephrin mRNA expression levels in the model group were higher than those in the control group. Nephrin mRNA expression levels were significantly lower in the intervention groups than that in the model group, especially the Chinese herb plus glucocorticoid group. Compared with the control group, the

model group showed increased nephrin expression in the kidney. Nephrin levels in the other groups, especially in the Chinese herb plus glucocorticoid group, were significantly lower than that in the model group. The nephrin levels in the kidney were negatively correlated with the proteinuria level.

CONCLUSION: Yiqiyangyin recipe could attenuate foot process injury particularly in combination with a steroid reduce the development of proteinuria possibly by inhibition of HPA in the kidney, and regulate the expression of nephrin in rats with ADR-induced nephropathy. Our study showed that treatment with Yiqiyangyin recipe plus glucocorticoid was better than a singular intervention, and we explored the pharmacological mechanism of this combination by biochemical and molecular biological analysis to provide a basis for clinical application.

Key words: Benefiting *Qi* for nourishing *Yin*; Chinese medical formula; Doxorubicin; Proteinuria; Heparanase; Nephrin

INTRODUCTION

Hormones are the preferred therapeutic agents for primary nephrotic syndrome (PNS), and can effectively control disease development. However, prolonged use of high doses of hormones often leads to obesity, developmental disorders, high blood pressure, diabetes, and other side effects. Therefore, it is of great clinical significance to seek new drugs and methods for improving the effects of hormones and reducing the adverse effects of hormones and other immunosuppressive agents. In recent years, Traditional Chinese Medicine (TCM) has been used in the treatment of kidney disease and has attracted attention at home and abroad, while the underlying therapeutic mechanism remains unclear.

In this study, we used an animal model of nephrotic syndrome (NS), and determined the protein to gene level expressions of heparanase (HPA) and nephrin to explore the effect of Chinese herb in an animal model.

MATERIALS AND METHODS

Animals and experimental design

Thirty male Sprague-Dawley (SD) rats, weighing 110-130 g, were purchased from and housed in the Department of Experimental Animal, Fudan University, and randomly divided into 5 groups: control, model, glucocorticoid, Chinese herb, and Chinese herb plus glucocorticoid groups. The rats were housed in a temperature- and humidity-controlled environment (20℃± 2℃, 50%-70%) on a normal 12 h light-dark cycle from 7∶00 am-7∶00 pm and maintained on regular chow and water ad libitum. Each rat received a single injection of doxorubicin hydrochloride 5 mg/kg via the tail vein. The rats in the control group received a single injection of normal saline via the tail vein.

From the 1st day after modeling, the animals were fed with some drug for 28 days: (a) control group, distilled water; (b) model group, normal feeding without intervention; (c) glucocorticoid group, prednisone (5 mg/kg^{-1} · day^{-1}); (d) Chinese herb group, Chinese herb granules (6.2 g/kg^{-1} · day^{-1}); and (e) Chinese herb plus glucocorticoid group, Chinese herb granules and prednisone at a dose same as above. Urine samples were collected for 24 h in the metabolic cages on days 0, 7, 14, 21, and 28 after the injection. The rats were killed on day 29. About 3 mL of blood was collected from the abdominal aorta. Further, left kidney cortex was divided into 1 mm × 1 mm × 1 mm in size, then, some were fixed with 10% neutral formalin for light microscopy, and the others were fixed with 3% glutaraldehyde for electron microscopy. The right kidney cortex was wrapped and stored in foil in liquid nitrogen for examination by real-time polymerase chain reaction (PCR).

Materials and reagents

Doxorubicin (Lot No. 8NB002-A) was purchased from Pharmacia & Upjohn, Inc. (Italy), prednisone (Lot No. Sinopharm quasi-word H31020675) from Shanghai Xinyi Pharmaceutical Factory Co., Ltd. (Shanghai, China), Chinese herbs (TCM granules, batch No. 0710081) from Sanjiu medical and pharmaceutical Co., Ltd. (Shenzhen, China), PCR Master Mix (Lot No. 75660M3) from Toyobo Company, Japan, rat HPA enzyme-linked immunosorbent assay (ELISA) Kit (Lot No. H2608) from TaKaRa Company, Japan, and rabbit anti-rat nephrin antibody (batch No. I1208) from Santa Cruz, California, US. The consists of Yiqiyangyin recipe were Dihuang (*Radix Rehmanniae*) 6 g, Shanyao (*Rhizoma Dioscoreae Oppositae*) 12 g, Mudanpi (*Cortex Moutan Radicis*) 6 g, Shenghuangqi (raw *Astragalus*) 9 g, Dangshen (*Radix Codonopsis*) 6 g, Danshen (*Radix Salviae Miltiorrhizae*) 6 g, Danggui (*Radix Angelicae Sinensis*) 6 g, Fuling (*Poria*) 9 g, Yimucao (*Herba Leonuri Japonici*) 4.5 g, Baizhu (*Rhizoma Atractylodis Macrocephalae*) 6 g and Guijia (*Chinemys reevesii Gray*) 4 g. Through boiling, filtration, the Chinese herb granules were concentrated for 0.62 g/mL by crude drug.

Protein concentration in the urine

The protein concentration in the urine for 24 h was measured by a colorimetric assay using Bio-Rad protein assay reagent and bovine serum albumin as a standard.

Blood biochemical index

The serum was separated from blood by centrifugation at normal temperature, and the serum indices (serum levels of creatinine, blood urea nitrogen, albumin, total cholesterol, and triglycerides) were measured using Hitachi 7060 automatic biochemical analyzer (Hitachi Ltd., Japan).

Light microscopic examination

Paraffin-embedded section of the rat kidney was modified through the renal hilum cross-sectional and paralleling short-axis. The slice containing the entire kidney section was examined by routine hematoxylin and eosin (HE) staining under the Eclipse TS100 light microscope (Nikon Instruments Inc., Japan) to observe the pathological changes in the kidney tissue.

Electron microscopic examination

Renal cortex was fixed using phosphate buffer (0.05/L, pH 7.2) containing 3% glutaraldehyde and 0.22 mmol/L sucrose, and 1% osmium tetroxide and then was fixed by ethanol dehydration and embedded using epoxy resin. Finally, the ultrastructure of the kidney was detected by Hitachi H-600 type transmission electron microscopy (Hitachi Ltd., Japan).

Real-time fluorescence quantitative PCR

Total RNA was extracted from the renal cortex using Trizol extraction method. Then, the cDNA was reverse transcribed from the total RNA using random hexanucleotide oligonucleotide polymers as primer and Promega's M-MLV reverse transcriptase. The SybrGreen kit was selected for real-time PCR. The primers were designed using primer premier 5.0 software (PREMIER Biosoft international, 3786 Corina Way, Palo Alto, CA, USA) and synthesized at the Shanghai Public Health and Bio-Engineering Co., Ltd. The primers and annealing temperatures were shown in Table 1. The amplification was performed using MX3000P (Agilent Technologies Inc., Santa Clara, CA, USA). The expression of mRNA was corrected using β-actin.

ELISA

The concentration of HPA in rat urine was measured by referring to protocol described in the kits. Since the level of HPA expression in the urine might be low, the supernatant was not diluted before testing.

Nephrin immunohistochemistry

Initially, paraffin sections were prepared and stained and then examined under Motic BA400 microscope (Motic China group Co., Ltd., Xiamen, China) at 400× to obtain micrographs. The results were analyzed using Motic image analysis 3.2 software (Motic China group Co., Ltd., Xiamen, China) and then average surface density (area of expression of nephrin protein and percentage of total area of vision) was calculated.

Statistical analysis

All data were analyzed using statistical software SPSS 13.0 (SPSS corporation, Chicago, USA) and expressed as mean±standard deviation (*SD*) ($\bar{x} \pm s$). Statistical significance was evaluated using the *t*-test and Pearson linear correlation analysis and $P<0.05$ was regarded as a significant difference.

RESULTS

Concentration of urinary protein for 24 h

The amount of 24-h urinary protein in the model group was higher than that in the control group 7 days after injection, but the increase was not statistically significant. Proteinuria increased on day 14 ($P<0.01$) in the model group and was the highest on day 28 (Table 2). Drug was used for 7 days from the first day after modeling. On day 7, the proteinuria in the intervention group was not significantly different from that in the model group. Proteinuria began to decrease on day 14 in the intervention group, especially in the Chinese herb plus glucocorticoid group, and the decrease was statistically significant ($P<0.01$). On day 21, proteinuria in the intervention group was significantly lower than that in the model group ($P<0.05$), and on day 28, proteinuria in other groups was significantly lower than that in the model group ($P<0.01$; Table 2).

Differences in blood biochemical indices in each group

Serum albumin: compared with the normal control group, the model group showed a significant reduction in the serum albumin levels on day 28 ($P<0.01$). How-

Table 1 Sequence of primers, length of products, and annealing temperatures

Gene	Sequence of primers (5′→3′)	Length of products (bp)	Annealing temperature (℃)
HPA	CTGGCTCTCTCTCCTGTTCAA ACATTATGGAGGTTCAGGACG	168	55
Nephrin	CCCCAACATCGACTTCACTT CTGGATGTTGGTGTGGTCAG	199	58
β-actin	TGACAGGATGCAGAAGGAGA TAGAGCCACCAATCCACACA	106	56

Note: HPA: Heparanase.

Table 2 Comparison of 24-h urinary protein levels at different time points among groups (mg/kg, $\bar{x} \pm s$)

Group	24-h urinary protein level			
	Day 7	Day 14	Day 21	Day 28
Control	86±39	90±43	84±43	98±40
Model	136±86	330±199[a]	650±351[a]	1030±786[a]
Chinese herb	124±70	213±62	447±185[b]	751±205[b]
Glucocorticoid	120±98	162±91[b]	389±56[c]	474±140[c]
Chinese herb plus glucocorticoid	130±8	155±62[b]	279±34[c]	377±76[c]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Compared with the control group at the same time point, [a]P<0.01; compared with the model group at the same time point, [b]P<0.05, [c]P<0.01.

ever, the serum albumin levels were higher in the intervention group than those in the model group, but the serum albumin levels in the glucocorticoid group and the Chinese herb plus glucocorticoid group showed a significant increase (P<0.01; Table 3).

Table 3 Differences in serum albumin levels among groups (g/L, $\bar{x} \pm s$)

Group	n	Serum albumin
Control	6	24.0±2.3
Model	6	19.6±3.9[a]
Chinese herb	6	22.4±1.2[b]
Glucocorticoid	6	25.9±0.5[c]
Chinese herb plus glucocorticoid	6	30.7±5.5[c]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Compared with the control group, [a]P<0.01; compared with the model group, [b]P<0.05, [c]P<0.01.

Serum creatinine and blood urea nitrogen: the serum creatinine and blood urea nitrogen levels were not significantly different between any two groups (all P> 0.05; Table 4).

Table 4 Differences in serum creatinine and blood urea nitrogen levels among groups ($\bar{x} \pm s$)

Group	n	Cr (μmol/L)	BUN (mmol/L)
Control	6	23.0±1.4	6.4±6.6
Model	6	30.4±4.2	7.3±0.9
Chinese herb	6	27.6±4.5	8.1±1.1
Glucocorticoid	6	27.3±2.3	7.4±0.5
Chinese herb plus glucocorticoid	6	28.3±3.2	8.0±0.8

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Cr: creatinine; BUN: blood urea nitrogen. No differences were found between any two groups, P>0.05.

Serum lipid: the levels of total cholesterol and triglycerides were significantly higher in the model group than those in the control group (P<0.05). Compared with the model group, the intervention groups, especially the Chinese herb plus glucocorticoid group, showed marked reduction in the levels of total cholesterol and triglycerides (P<0.05; Table 5).

Table 5 Differences in serum lipid levels among groups (mmol/L, $\bar{x} \pm s$)

Group	n	Total cholesterol	Triglyceride
Control	6	1.20±0.08	0.90±0.07
Model	6	1.63±0.44[a]	1.35±0.11[a]
Chinese herb	6	1.30±0.32	1.20±0.37
Glucocorticoid	6	1.29±0.16	1.09±0.18[b]
Chinese herb plus glucocorticoid	6	1.15±0.13[b]	1.08±0.28[b]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Compared with the control group, [a]P<0.05; compared with the model group, [b]P<0.05.

Pathological changes in kidney tissue under light microscope

The kidney of the rats in the normal control group showed no pathological changes. The rats in the model group showed a slight increase in glomerular volume. Mesangial cells and matrix showed a mild increase in focal and segmental parts, but necrosis, crescents, and sclerosis were not observed in the kidney. The renal tubular epithelial cells showed multifocal granular and vacuolar degeneration but did not show any significant tubular atrophy. The cells occasionally showed protein casts. The renal interstitium showed inflammatory cell infiltration simultaneously with a small amount of fibrous tissue hyperplasia. The intima of some small arteries was thickened. Compared with the model group, the intervention groups all showed an improvement in the pathological changes in the renal tissue and reduc-

tion in the inflammatory cell infiltration in the renal interstitium (Figure 1).

Ultrastructural changes in kidney under electron microscope

In the control group, glomerular basement membrane was smooth and uniform, and no thickening was observed. The feet were clear and complete without fusion. The rats in the model group showed occasional thickening of the glomerular basement membrane, but majority of the feet showed fusion and disappearance or the presence of microvilli on the feet. The microscopic appearance of the model group was significantly different from that of the control group. The glomerular basement membrane in the Chinese herb group was smooth and uniform basically, feet did not show fusion, and growth of a small amount of microvilli was observed. In the glucocorticoid group and the Chinese herb plus glucocorticoid group, feet were clearly similar to their appearance in the control group (Figure 2).

HPA expression in each group

HPA mRNA expression in renal tissue: HPA mRNA level was significantly higher in the model group than that in the control group (P<0.001), meanwhile, its level in the Chinese herb group and the Chinese herb plus glucocorticoid group was lower than that in the model group, and the difference was statistically significant (P<0.05; Table 6).

HPA concentration in urine: the urinary HPA concentration in the model group was significantly higher than that in the control group (P<0.05), while the HPA concentration in the intervention groups was lower than that in the model group and higher than that in the control group, but the difference was not statistically significant (P>0.05; Table 7).

Correlation analysis: the HPA mRNA concentration in urine and the amount of 24-h urinary protein was significantly positively correlated (r=0.708, P<0.005) (Figure 3).

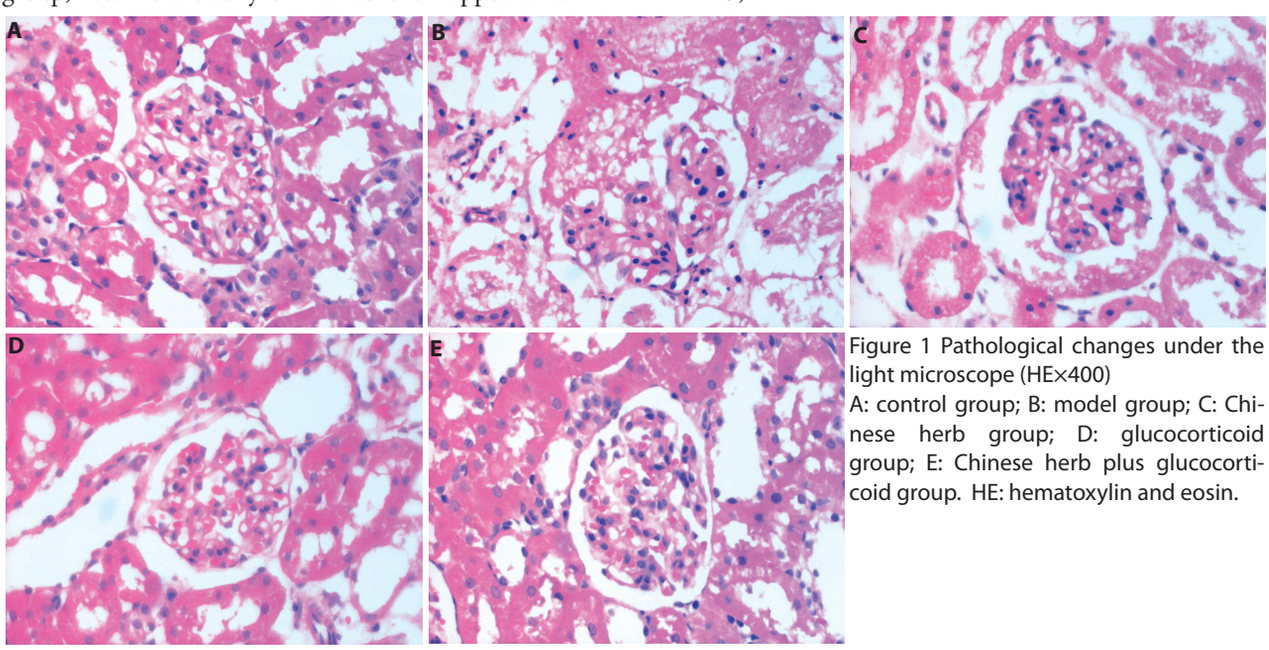

Figure 1 Pathological changes under the light microscope (HE×400)
A: control group; B: model group; C: Chinese herb group; D: glucocorticoid group; E: Chinese herb plus glucocorticoid group. HE: hematoxylin and eosin.

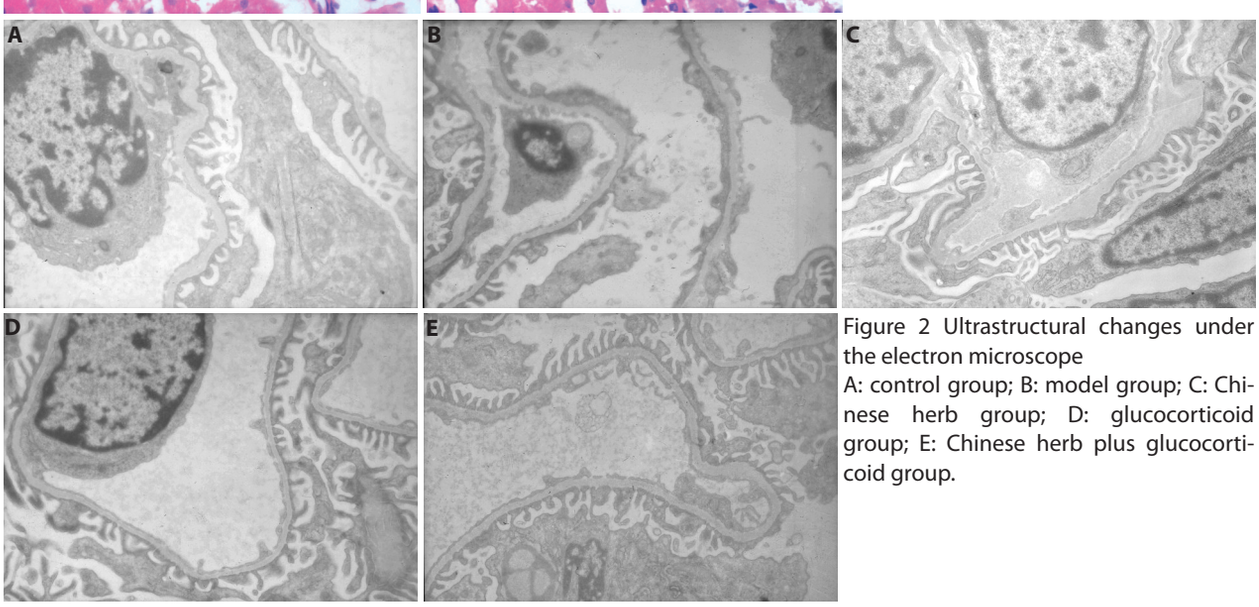

Figure 2 Ultrastructural changes under the electron microscope
A: control group; B: model group; C: Chinese herb group; D: glucocorticoid group; E: Chinese herb plus glucocorticoid group.

Table 6 Comparison of heparanase mRNA expression among groups ($\bar{x}\pm s$)

Group	*n*	HPA mRNA
Control	6	1.50±0.79
Model	6	4.55±0.38[a]
Chinese herb	6	3.56±0.18[b]
Glucocorticoid	6	3.99±0.35
Chinese herb plus glucocorticoid	6	3.13±0.81[b]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. HPA: heparanase. Compared with the control group, [a]*P*<0.001; compared with the model group, [b]*P*<0.05.

Table 7 Comparison of HPA concentration in urine among groups (pg/mL, $\bar{x}\pm s$)

Group	*n*	HPA
Control	6	5.04±0.61
Model	6	6.20±1.00[a]
Chinese herb	6	5.47±0.41
Glucocorticoid	6	5.39±0.78
Chinese herb plus Glucocorticoid	6	5.65±0.28

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. HPA: heparanase. Compared with the control group, [a]*P*<0.05.

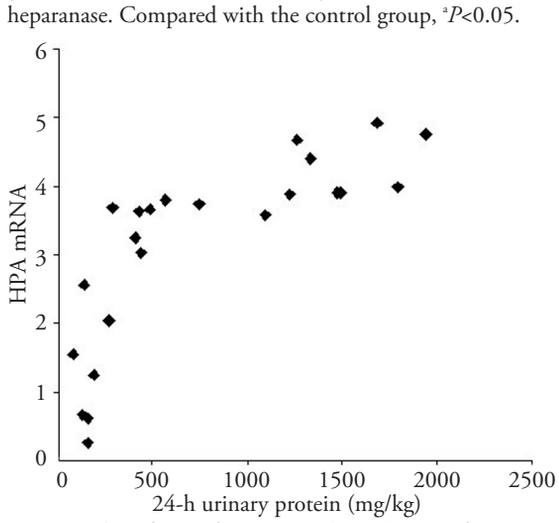

Figure 3 Correlation between HPA mRNA and amount of 24-h urinary protein
HPA: heparanase.

Nephrin expression in each group

Nephrin mRNA expression in renal tissue: the nephrin mRNA expression was significantly higher in the model group than that in the control group (*P*<0.05). The expression of nephrin in the intervention groups, especially in the glucocorticoid group and the Chinese herb plus glucocorticoid group, showed a significant decrease and was close to the normal expression levels (*P*=0.03<0.05; Table 8).

Table 8 Comparison of nephrin mRNA expression among groups ($\bar{x}\pm s$)

Group	*n*	Nephrin mRNA
Control	6	0.93±0.07
Model	6	1.04±0.11[a]
Chinese herb	6	0.96±0.11
Glucocorticoid	6	0.92±0.02[b]
Chinese herb plus Glucocorticoid	6	0.91±0.01[b]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Compared with the control group, [a]*P*<0.05; compared with the model group, [b]*P*<0.05.

Nephrin expression in renal tissue: compared with the control group, the model group showed a significant decrease in the nephrin expression (*P*<0.05). Furthermore, the nephrin expression was particularly significantly higher in the Chinese herb plus glucocorticoid group than that in the model group (*P*=0.016<0.05) (Table 9, Figure 4).

Table 9 Comparison of nephrin expression at protein level among groups (%, $\bar{x}\pm s$)

Group	*n*	Nephrin
Control	6	23.8±4.5
Model	6	15.1±1.4[a]
Chinese herb	6	17.0±1.0
Glucocorticoid	6	18.5±1.6
Chinese herb plus glucocorticoid	6	19.9±3.3[b]

Notes: Control group treated with distilled water; model group treated with normal feeding without intervention; glucocorticoid group treated with prednisone; Chinese herb group treated with Chinese herb granules; Chinese herb plus glucocorticoid group treated with Chinese herb granules and prednisone. Compared with the control group, [a]*P*<0.05; compared with the model group, [b]*P*<0.05.

Correlation analysis: the nephrin expression in the renal tissue and the 24-h urinary protein concentration were negatively correlated (*r*=-0.66, *P*=0.001) (Figure 5).

DISCUSSION

NS comprises excessive proteinuria, edema, and hypercholesterolemia as marked manifestations. In the TCM viscera theory, the etiology and pathogenesis of NS are correlated with the innate deficiency for some children in Lung, Spleen and Kidney,[1,2] or weakness due to chronic diseases, and exogenous pathogens invasion. The dysfunction of these organs for *Qi* transformation and fluid regulation resulted in stagnation of water and dampness and essence or leakage of protein loss from urine. For NS in children, the asthenia of Lung, Spleen and Kidney are essential or "Ben", exogenous dampness or damp-heat and inner retention of Water and

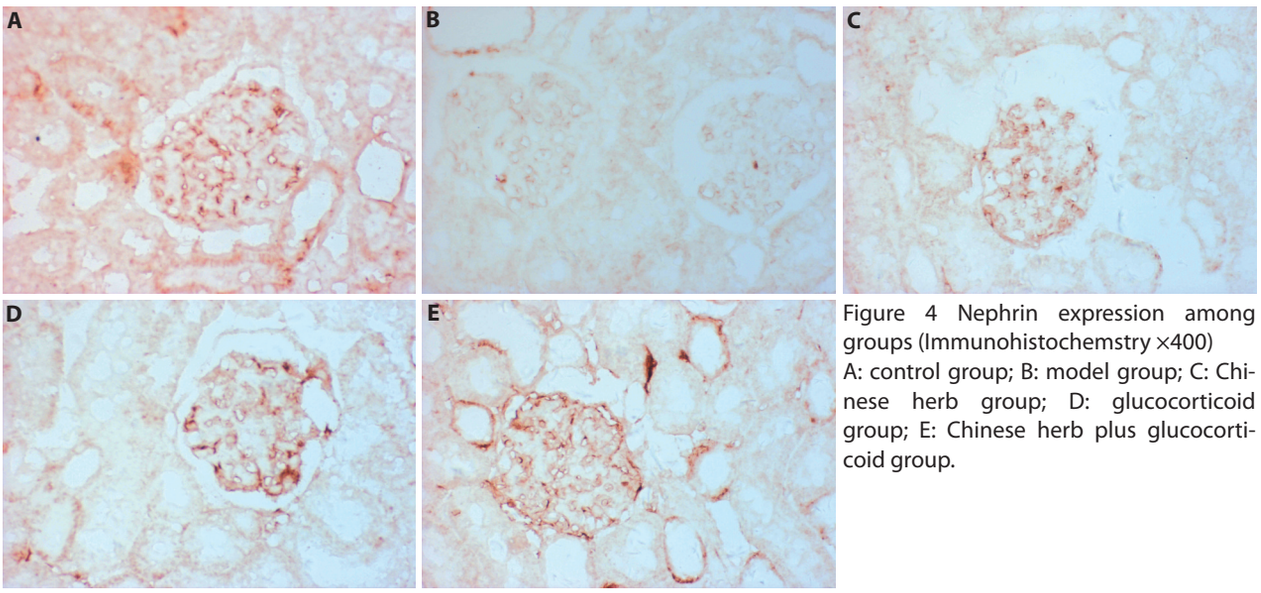

Figure 4 Nephrin expression among groups (Immunohistochemstry ×400)
A: control group; B: model group; C: Chinese herb group; D: glucocorticoid group; E: Chinese herb plus glucocorticoid group.

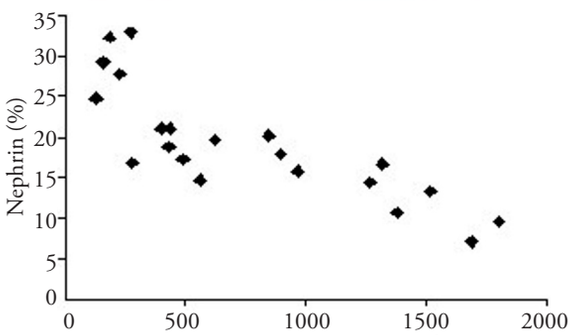

Figure 5 Correlation between the levels of nephrin and 24-h urinary protein

Dampness are sthenia or "Biao" syndrome.

Yiqiyangyin recipe is a Chinese herb mixture commonly used in our outpatient department and integrative medicine ward. Therapeutic effects of Yiqiyangyin recipe include nourishing the kidney, invigorating the spleen, reinforcing *Qi*, nourishing *Yin*, promoting blood, and removing dampness, etc. Yiqiyangyin recipe has shown good clinical results. In children with repeated and low-dose steroid-dependent NS, the herbs can help in gradual reduction of the steroid dose.

Renal glomerular filtration barrier is one of the important structures, and the integrity of barrier determines the permeability characteristics for the protein. Glomerular filtration barrier is divided into 3 layers: fenestrated endothelial cell layer, glomerular basement membrane (GBM), podocyte, and slit diaphragm (SD). The GBM, podocyte, and SD prevent the protein leakage. The GBM mainly forms a charge barrier, while the podocytes and SD form a barrier dependent on pore size. Damage to the structure and function of the glomerular filtration barrier can lead to protein leakage. In patients with minimal change nephropathy, changes in pore size and charge barriers have been reported.[3] Recently, the role of HPA in proteinuria has attracted widespread attention.[4,5] HPA can degrade the heparin sulfate proteoglycan (HSPGs) of the HS side chain in GBM. Thus, change in expression and distribution of HSPGs in the GBM can lead to change in GBM charge barrier and molecular barrier dysfunction, thereby affecting the glomerular filtration function.

In an animal model of puromycin (PAN)-induced minimal-change nephropathy (MCD), Levidiotis *et al* [6] found proteinuria after 5 days and massive proteinuria appeared after 14 days. By means of the Western blot, the expression of 65 kD HPA was increased on the 5th day of the onset, and the expression of 58 kD HPA with the active form was visible on the 14th day of the onset. Northern hybridization showed a significant increase in the expression of glomerular HPA mRNA 14 days after modeling, and a highly active form of the 2.0 kb mRNA-based, which prompts in glomeruli of PAN nephropathy, increased HPA expression, and participate in proteinuria generation. The PAN model was characterized by podocyte changes and showed that the glomerular podocyte activation may be a potential mechanism for the activation of HPA expression. In a doxorubicin model, Luo *et al* [7] detected the HPA gene expression in the peripheral blood of rats by semi-quantitative RT-PCR, and their results showed that the level of proteinuria in the model group was significantly higher than that in the normal control group. A recent study showed that treatment ofADR-induced nephropathy with an angiotensin II (Ang II) receptor antagonist in rats reduced the HPA expression and proteinuria, while maintained the HS expression in the GBM.[8] In addition, Shafat *et al* [9] found that urinary heparanase was markedly elevated and associated with proteinuria in chronic kidney disease (CKD) patients.

Our experimental study showed that 28 days after modeling, the HPA mRNA expression in the renal tissue (4.55 ± 0.38) and HPA level in the urine (6.23 ± 1.10) in the model group were significantly higher than those in the control group (P>0.05), which is consistent with the findings reported in previous studies.[7] After 28 days, the expression of HPA mRNA in the renal tissue in the Chinese herb plus glucocorticoid group (5.65±0.28) was significantly lower than that in the model group. In each group, the HPA mRNA ex-

pression and the level of proteinuria were positively correlated (r=0.708, P=0.005). Thus, proteinuria is suggested to be related to the HPA expression, especially in the intervention groups. Furthermore, the HPA concentration in the urine in the intervention groups was lower than that in the model group but higher than that in the control group. Thus, the reduction in the HPA concentration may occur on a later stage in the urine than that in the kidney.

As mentioned earlier, the glomerular filtration barrier consists of fenestrated endothelial cells, GBM, and SD among the foot processes of the cell. The impact of the protein filtration barrier is the SD in the foot process membrane. Nephrin, which belongs to the immunoglobulin superfamily, is a transmembrane protein and an important component of the SD. A study showed loss of renal nephrin for the first time in patients with congenital nephrotic syndrome[10] and then in a model of PNS[11] and PAN[12] ,which suggested development of nephrotic proteinuria and abnormalities in nephrin expression. In addition, nephrin contains more anions, may be by the side chains of sugar nephrin into the glycosaminoglycan sulfate, heparin and other offers such as sulfuric acid, which suggests that nephrin may have a charge barrier. Therefore, nephrin is an important component of the SD, and may play a key role in maintaining the glomerular filtration barrier.

We found that the expression of mRNA was higher in the model group than in the control group (P<0.05), which was consistent with the study result by Peng *et al*.[13] However, in our study, compared with the control group, the model group showed a significant decrease in the average surface density ratio of the kidney (P< 0.05). Therefore, we believed that the expressions of nephrin protein and mRNA were inconsistent or even opposite.

Many previous studies have not shown consistent results about nephrin expression. Luimula *et al* [14] observed that the expression of nephrin mRNA decreased in puromycin nephropathy and Heymann nephritis model. Furness *et al* [15] showed similar results in minimal change disease and membranous nephropathy. However, Schaefer *et al* [16] found that the nephrin expression increased in anti-Thy1.1 nephritis in rats. Guan *et al* [17] and Fan *et al* [18] obtained similar results to Schaefer *et al*. Thus, the difference in the nephrin expression may be closely related to the development of proteinuria, models, and testing methods, etc.

We observed different degrees of reduction in nephrin mRNA expression in each intervention group, especially in the glucocorticoid group and the Chinese herb plus glucocorticoid group (P=0.03<0.05). The average surface density ratio of nephrin was lower in the model group than those in the intervention groups. There were statistically significance in the Chinese herb plus glucocorticoid group compared with the model group for nephrin gene expression (P=0.016<0.05). These results suggest that the Chinese herb could reduce the proteinuria. Meanwhile, the Chinese herb plus glucocorticoid group had more significant effects, and this may be the theoretical basis of treating NS using Chinese herb plus glucocorticoid.

REFERENCES

1 **Zheng J**. Clinical thought of treatment of pediatric primary nephrotic syndrome based on syndrome differentiation. Zhong Hua Zhong Yi Yao Za Zhi 2007; 22(4): 225-227.

2 **Ou Yang HP**, Si ShL, Wu XL, Wang MH. Clinical research of TCM therapy on spleen and kidney dysfunction of Primary nephrotic syndrome. Zhong Yi Yao Xue Bao 2012; 40(1): 84-87.

3 **Niels W P Rutjes**, Nike Claessen, Jeroen KJ Deegens, et al. The role of heparanse in the pathophysiology of idiopathic nephritic syndrome. Nephrology 2005; 10(suppl): A117-A232.

4 **Zcharia E**, Metzger S, Chajek-ShaulL T, et al. Transgenic expression of mammalian heparanase uncovers physiological functions of heparan sulfate in tissue morphogenesis, vascularization, and feeding behavior. FASEB J 2004; 18 (2): 252-263.

5 **Kramer A**, van den Hoven M, Rops A, et al. Induction of glomerular heparanase expression in rats with adriamycin nephropathy is regulated by reactive oxygen species and the renin-angiotensin system. J Am Soc Nephrol 2006; 17 (9): 2513-2520.

6 **Levidiotis V**, Freeman C, Tikellis C, et al. Heparanase is involved in the pathogenesis of proteinuria as a result of glomerulonephritis. J Am Soc Nephrol 2004; 15(1): 68-78.

7 **Luo W**, Wang ZH. Gene expression of Xylosyltransferase and Heparanase in renal tissue of rat with Adriaamycin nephropathy and relevance for proteinuria. Sichuan Da Xue Xue Bao (Medical Science Edition) 2007; 38(6): 965-958.

8 **Kramer A**, van den Hoven M, Rops A, et al. Induction of glomerular heparanase expression in rats with adriamycin nephropathy is regulated by reactive oxygen species and the renin-angiotensin system. J Am Soc Nephrol 2006; 17 (9): 2513- 2520.

9 **Shafat I**, Agbaria A, Boaz M, et al. Elevated urine heparanase levels are associated with proteinuria and decreased renalallograft function. PLoS ONE 2012; 7(9): e44076.

10 **Marjo Kestilä**, Ulla Lenkkeri, Minna Männikkö, et al. Positionally cloned gene for a novel glomerular protein-Nephrin-is mutated in Congenital Nephrotic Syndrome. Molecular cell 1998; 1(4): 575-582.

11 **Doublier S**, Ruotsalainen V, Salvidio G, et al. Nephrin redistribution on Podocytes is a potential mechanism for proteinuria in patients with primary acquired nephritic syndrome. Am J Pathol 2001; 158(5): 1723-1731.

12 **Luimula P**, Ahola H, Wang SX, et al. Nephrin in experimental glomerular disease. Kidney Int 2000; 58(4): 1461-1468.

13 **Peng YQ**, Mo Y, Jiang XY, et al. Expression and significance of Nephrin ang CD2AP in adriamycin-induced nephrotic rats. Zhong Shan Da Xue Xue Bao (medicine sci-

ences) 2009; 30(1): 15-20.

14 **Luimula P**, Sandstrom N, Novikov D, Holthöfer H. Podocyte-associated molecules in puromycin aminonucleoside nephrosis of the rat. Lab Invest 2002; 82(6): 713-718.

15 **Furness PN**, Hall LL, Shaw JA, et al. Glomerular expression of nephrin is decreased in acquired human nephrotic syndrome. Nephrol Dial Transplant 1999; 14(5): 1234-1237.

16 **Schaefer L**, Ren S, Schaefer RM, et al. Nephrin expression is increased in anti- Thy1. 1-induced glomerulonephritis in rats. Biochem Biophys Res Commun 2004; 324 (1): 247-254.

17 **Guan N**, Ding J, Zhang JJ, et al. Relationship between expression of nephrin, podocin and α-actinin-4 with proteinuria in puromycin aminonucleoside nephrosis rats. Zhong Hua Shen Zang Bing Za Zhi 2004; 20(1): 26-32.

18 **Fan QF**, Ding J, Zhang JJ, et al. Influece of the sequence specific knockdown of podocin mRNA on the expression of nephrin and α-actinin in mouse podocyte. Zhong Hua Shen Zang Bing Za Zhi 2004; 10(5): 325-329.